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Improvement of marine environmental pollution using eco-system: decomposition and recovery of endocrine disrupting chemicals by marine phyto- and zooplanktons

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Dedicated to Professor Dr. Kenji Soda in honour of his 70th birthday

Abstract

The decomposition and the recovery of endocrine disrupting chemicals (EDCs) using marine phytoplankton were demonstrated as one of the possible bioremediation methods. Bis(2-ethylhexyl)phthalate and bisphenol A tended to gradually accumulate into the plankton cells during incubation. Furthermore, the recovery of bisphenol A from the synthetic seawater was achieved using a marine pollutant collecting model (eco-system) that combined phyto- and zooplanktons.

Keywords: Endocrine disrupting chemicals; Phytoplankton; Zooplankton; Eco-system; Marine pollution

1. Introduction

There is great concern about the possible harmful consequences of exposure to xenobiotic compounds that are capable of disrupting the endocrine system. Such compounds that are present in the aquatic environment and cause abnormal endocrine function in wildlife populations have been termed endocrine disrupting chemicals (EDCs) [1,2]. The impact and effect of these EDCs on the reproduction and development of vertebrates have been shown to be significant in many field and experimental studies [3–7]. Over the past decade, the number of investigations into the impact and effect of EDCs that affect reproductive and sexual characteristics has increased and evidence of their potency is obvious in numerous wildlife species and through data from in vitro tests [8–10].

Bioremediation is a rapidly developing field of environmental restoration, utilizing natural microbial activity to reduce the concentration and/or toxicity of various chemical substances, such as EDCs. Biodegradation is a natural process carried out by soil and aquatic microorganisms, mostly bacteria and fungi. Certain bacterial strains have a demonstrated ability to break down or transform these chemical substances (e.g. [11–13]). However, little information is known about the bioremediation for marine environmental pollutants.

In this paper, we report the recovery of marine pollutants, mainly synthetic endocrine disruption related substances, using marine plankton as an improvement method of the marine environment by bioremediation.

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2. Materials and method

2.1. Instruments

Gas chromatography was performed using a GL Science GC-353 (capillary column: TC-1, GL Science, Japan, 0.25 mm × 30 m) gas chromatograph.

2.2. Materials

Bis(2-ethylhexyl)phthalate (**1**), bisphenol A (*p,p′*-isopropylidenediphenol) (**2**), and *p*-*tert*-octylphenol (*p*-(1,1,3,3-tetramethylbutyl)phenol) (**3**) were purchased from Wako Pure Chemicals, Japan. 4-*n*-Octyloxyphenol (**4**) was purchased from Tokyo Kasei Kogyo, Japan (Scheme 1). Extrelut® was purchased from Merck, Germany. *Chaetoceros gracilis* (EPFES-YU-1, phytoplankton), *Nannochloropsis* sp. (EPFES-YU-3, phytoplankton), and *Artemia* sp. (zooplankton, EPFES-YU-5) were type cultures from Ehime Prefectural Fisheries Experimental Station, Uwazima, Ehime, Japan. *Brachinous* sp. (zooplankton) was kindly donated by Dr. Isao Maruyama, Research Laboratories, Chlorella Industries Co., Ltd., Fukuoka, Japan. All other chemicals and solvents used in this study were of analytical grade and commercially available.

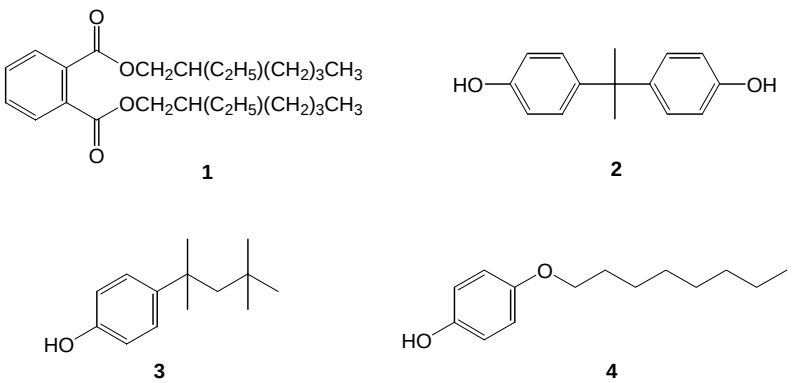
2.3. Growth medium and cultivation

The marine algae were photoautotrophically cultivated in synthetic seawater (1 l) for 2 weeks at 20 °C with constant aeration by air (2 l/min) in baffled 1 l flasks with illumination by white fluorescent light (1000 lx, one side). The synthetic seawater is comprised of 20.747 g NaCl, 0.8 μg $MnCl_2{\cdot}4H_2O$, 9.474 g $MgCl_2{\cdot}6H_2O$, 1.326 g $CaCl_2{\cdot}6H_2O$, 3.505 g Na_2SO_4, 597 mg KCl, 171 mg $NaHCO_3$, 85 mg KBr, 34 mg $Na_2B_4O_7{\cdot}10H_2O$, 12 mg $SrCl_2$, 3 mg NaF, 1 mg LiCl, 0.07 mg KI, 0.2 μg $CoCl_2{\cdot}6H_2O$, 8 μg $AlCl_3{\cdot}6H_2O$, 5 μg $FeCl_3{\cdot}6H_2O$, 0.2 μg $Na_2WO_4{\cdot}2H_2O$, 0.02 mg $(NH_4)_6Mo_7O_{24}$, and 1.0 ml of NM solution per 1 l of distilled water. The NM solution is a vitamin solution: $NaNO_3$ (150 g), Na_2HO_4 (10 g), EDTA-2Na (0.9 g), vitamin B_{12} (1.5 mg), thiamine·HCl (75 mg), biotin (1 mg), EDTA-Fe (2.5 g), and $H_2NC(CH_3OH)_3$ (5 g) were dissolved in 1 l of distilled water.

Chaetoceros was grown in the synthetic seawater containing 0.0045% sodium silicate (Na_2SiO_3). The wet cells were collected by centrifugation at 8000 × *g* for 15 min (about 2.0 g of wet cells was obtained from 500 ml of the each cultures). Zooplanktons (*Artemia* sp. and *Brachinous* sp.) were cultivated at 20 °C with constant aeration by air (2 l/min) in the synthetic seawater (500 ml) containing pre-cultured *Nannochloropsis* sp. as a feed.

2.4. Reaction with marine microalgae

The seawater-washed cells (about 0.5 g) were resuspended in a large test tube (ϕ 30 mm × 200 mm) containing 50 ml of the seawater; then the substrate (1.2 μmol, 24 μM) was added and incubated at 20 °C with aerobic shaking under illumination by white



Scheme 1.

fluorescent light (1000 lx, one side). The used cells were filtrated on a glass filter and washed with the flesh seawater, and then were suspended in the flesh seawater. This suspension was cooled at 4 °C and subsequently sonicated with 10 pulses of 120 s each with 300 s cooling intervals in a Sonicator® (Ohtake Works, Japan), fitted with a microtip at a power setting of 70 W. The intracellular substrate was extracted from the homogenated cells with diethyl ether (Fraction-A, incorporated substrate into the cells). The remained substrate in the used medium was extracted twice with diethyl ether from the used medium and the cell-washed seawater (Fraction-B, the remained substrate in the used medium). The extracts were concentrated under reduced pressure.

2.5. Bio-concentration with "eco-system"

The eco-system bioreactor was composed of the following reactor; an acrylic resin pipe (ϕ, 80 mm × 100 mm) equipped with nylon mesh (100 mesh) at the bottom of the pipe attached inside a 2 l flask as shown in Fig. 1.

To the eco-system reactor, the cultivated phytoplankton (*Nannochloropsis* sp. 1.8 l suspension, 2 weeks cultivated) was added, and then the cultivated zooplankton (*Artemia* sp. or *Brachinous* sp., 100 ml suspension, after 1 week cultivated) was added to the inside the acrylic pipe. Finally, the substrate (3 μmol per acetone solution) added to the outside flask and and incubated at 20 °C with gentle stirring under illumination by white fluorescent light (1000 lx, one side). Fig. 2 is a schematic diagram of the eco-system.

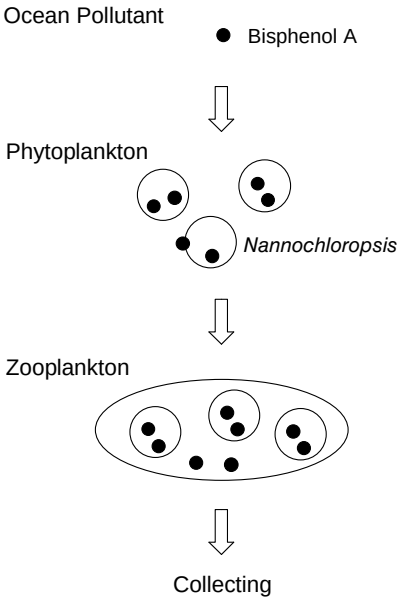


Fig. 2. Schematic diagram of the eco-system.

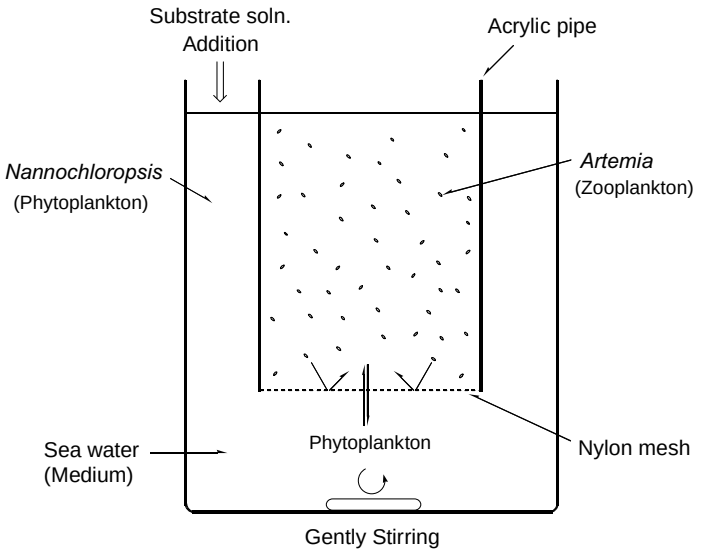


Fig. 1. The marine pollutant collecting model using the "eco-system".

2.6. Analysis

The amounts of the substrate in the used cells and the used medium were measured by GLC equipped with a capillary TC-1 column with the internal standard (**1**,**2**,**4**: *n*-hexadecane, **3**: *n*-tetradecane). The recovery (%) of the substrate was calculated by setting the initial amount without incubation to 100.

3. Results and discussion

3.1. The recovery of bis(2-ethylhexyl)phthalate and bisphenol A

As shown in Tables 1 and 2, the recoveries (%) of the substrates (**1** and **2**) in the used medium decreased during incubation with the marine microalgae, while the amounts of the substrates in the algal cells increased. These results indicate that the administered substrate gradually became incorporated and accumulated into the algal cells.

3.2. The recovery of p-tert-octylphenol and 4-n-octyloxyphenol

The amounts of the substrate in the cells (Fraction-A) were scarcely changed during the incubation, however, the recoveries (%) of the substrates (**3** and **4**) in the medium and the total recoveries of the substrates tended to decrease (see Tables 3 and 4). These results suggest that the administered substrates immediately decompose after incorporation into the algal cells and the decomposition preferentially occurs versus the accumulation into the cells.

3.3. The recovery of bisphenol A using "eco-system"

The recovery experiment of bisphenol A (**2**) from seawater was carried out using a marine pollutant collecting model as shown in Fig. 1. When only using phytoplankton (*Nannochloropsis* sp.), the administered substrate remained 11% in the medium and was 46% recovered from the phytoplankton cells (see Table 5). Almost all of the substrate remained in the medium using only zooplankton (*Artemia* sp. or *Brachionus* sp.). Over 40% of the substrate was recovered from the zooplankton cells using a combination of the phyto- and zooplanktons, i.e. "an eco-system". These results suggest that the substrate migrated and accumulated in the zooplankton cells through the phytoplankton cells.

Thus, we have been demonstrated the decomposition and the recovering of EDCs by marine phyto- and/or zooplanktons. In particular, the recovery of bisphenol A from seawater using the eco-system presented in this study would become one of the effective methods for the recovery of diluted marine pollutants over a wide area such as an ocean.

Table 1
The recovery (%) of **1** diring the reaction with marine microalgae

Marine algae	After 1 day		After 2 days		After 6 days	
	Frac.-A	Frac.-B	Frac.-A	Frac.-B	Frac.-A	Frac.-B
Nannochloropsis sp.	12	61	19	44	26	33
C. gracilis	38	57	38	33	38	14

Frac.-A: Fraction-A (recovery of substrate in the used algal cells). Frac.-B: Fraction-B (recovery of substrate in the used medium).

Table 2
The recovery (%) of **2** during the reaction with marine microalgae

Marine algae	After 1 day		After 2 days		After 6 days	
	Frac.-A	Frac.-B	Frac.-A	Frac.-B	Frac.-A	Frac.-B
Nannochloropsis sp.	14	68	49	24	53	13
C. gracilis	15	56	23	43	25	34

Frac.-A: Fraction-A (recovery of substrate in the used algal cells). Frac.-B: Fraction-B (recovery of substrate in the used medium).

Table 3
The recovery (%) of **3** during the reaction with marine microalgae

Marine algae	After 1 day		After 2 days		After 6 days	
	Frac.-A	Frac.-B	Frac.-A	Frac.-B	Frac.-A	Frac.-B
Nannochloropsis sp.	2	34	3	24	4	15
C. gracilis	2	21	4	17	5	11

Frac.-A: Fraction-A (recovery of substrate in the used algal cells). Frac.-B: Fraction-B (recovery of substrate in the used medium).

Table 4
The recovery (%) of **4** during the reaction with marine microalgae

Marine algae	After 1 day		After 2 days		After 6 days	
	Frac.-A	Frac.-B	Frac.-A	Frac.-B	Frac.-A	Frac.-B
Nannochloropsis sp.	23	17	19	13	4	11
C. gracilis	66	15	19	13	5	9

Frac.-A: Fraction-A (recovery of substrate in the used algal cells). Frac.-B: Fraction-B (recovery of substrate in the used medium).

Table 5
The recovery (%) of **2** during the reaction with the phyto- and zooplankton "eco-system"

Plankton	Recovery (%)		
	Zooplankton	Phytoplankton	Medium
Artemia sp. *Nannochloropsis* sp.	41	9	9
Brachionus sp. *Nannochloropsis* sp.	44	10	10
Nannochloropsis sp.	–	46	11
Artemia sp.	5	–	83
Brachionus sp.	6	–	80

To gain insight into the mechanistic interpretation of the recovery and accumulation using phyto- and zooplanktons, further detailed studies including purification of the related enzyme(s) are currently under investigation. The application of the presented method for the field under realistic conditions is also now in progress in our laboratory.

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